

09/76,085

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l3 and solid	▲
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<u>L4</u>	l3 and solid	14	<u>L4</u>
<u>L3</u>	L2 and chelat\$	14	<u>L3</u>
<u>L2</u>	L1 and chaotrop\$ and divalent	19	<u>L2</u>
<u>L1</u>	(purif\$ or isolat\$ or separat\$) near5 single stranded	1328	<u>L1</u>

END OF SEARCH HISTORY

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- ☐ 1. [6309822](#). 23 Dec 96; 30 Oct 01. Method for comparing copy number of nucleic acid sequences. Fodor; Stephen P. A., et al. 435/6; 435/287.2 435/288.3 435/288.7 536/23.1 536/24.3 536/24.31. C12Q001/68 C12M001/34 C07H021/04 C07H021/02.
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- ☐ 3. [5958689](#). 21 May 97; 28 Sep 99. Detection of toxigenic marine diatoms of the genus *Pseudo-nitzschia*. Scholin; Christopher A., et al. 435/6; 536/23.1 536/24.3. C12Q001/68 C07H021/02 C07H021/04 C12N015/00.
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- ☐ 4. [5837500](#). 03 Apr 95; 17 Nov 98. Directed evolution of novel binding proteins. Ladner; Robert Charles, et al. 435/69.7; 435/471 435/91.1 435/91.2 530/350 530/412 536/23.4. C12N015/62 C07K019/00.
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- ☐ 7. [5726021](#). 27 Nov 96; 10 Mar 98. Rapid and sensitive detection of antibiotic-resistant mycobacteria using oligonucleotide probes specific for ribosomal RNA precursors. Britschgi; Theresa B., et al. 435/6; 435/259 435/4. C12Q001/68 C12Q001/00.
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- ☐ 9. [5571698](#). 18 Jun 93; 05 Nov 96. Directed evolution of novel binding proteins. Ladner; Robert C., et al. 435/69.7; 435/252.3 435/320.1 435/477 435/6 435/69.1. C12N025/62.
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- ☐ 10. [5403484](#). 26 Jan 93; 04 Apr 95. Viruses expressing chimeric binding proteins. Ladner; Robert C., et al. 435/235.1; 435/252.3 435/320.1 435/69.7 530/350 536/23.4. C07K013/00 C12N007/01.
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L4: Entry 5 of 14

File: USPT

Sep 8, 1998

DOCUMENT-IDENTIFIER: US 5804684 A

TITLE: Method for isolating nucleic acids

Abstract Text (1):

The invention features a method of isolating nucleic acid in a substantially purified form, including the steps of: a) contacting a biological sample which contains aggregated nucleic acid with a matrix comprising a solid hydrophilic organic polymer without an effective positive charge under conditions which permit the nucleic acid to bind to the matrix; and b) recovering nucleic acid from the matrix.

Brief Summary Text (5):

U.S. Pat. No. 5,346,994 to Chomczynski discloses a liquid nucleic acid isolation method employing a reagent mixture of phenol, chaotropic salts and stabilizers. The procedure involves cell lysis and separation of DNA, RNA and proteins in different phases by centrifugation.

Brief Summary Text (10):

One object of the invention is to isolate nucleic acid from a biological sample in a simple, fast, and efficient process. Another object is to provide a method which avoids high speed centrifugation and phase separation to isolate a nucleic acid. Another object is to provide for highly quantitative recovery of nucleic acid regardless of its molecular weight. Another object of the invention is to isolate nucleic acid from a number of samples simultaneously, thus saving time and effort and providing for subsequent simultaneous processing of the samples. Yet another object of the invention is to provide for simultaneous processing and recovery of even small amounts of nucleic acids from multiple samples. Another object of the invention is to avoid the risk of loss of an isolated nucleic acid by providing a nucleic acid preparation which does not require further concentration from a large volume or does not require further purification. Yet another object of the invention is to provide for high yield recovery of nucleic acid within a broad size range. Another object is to provide a method of nucleic acid isolation that is environmentally friendly, i.e., that avoids the use of toxic chemicals, corrosive agents or chaotropic salts.

Brief Summary Text (12):

The invention is based on a method and apparatus for nucleic acid isolation. The invention utilizes the properties of aggregated nucleic acids to isolate and purify nucleic acids from contaminants such as other cellular components. The invention is based on the discovery that aggregated nucleic acid is capable of binding reversibly to a solid, hydrophilic organic matrix without an effective positive charge.

Brief Summary Text (13):

The invention therefore encompasses a method of isolating nucleic acid in a substantially purified form, the method comprising the steps of: a) contacting a biological sample comprising aggregated nucleic acid with a matrix under conditions which permit nucleic acid in the sample to reversibly bind to the matrix, the matrix comprising a solid hydrophilic organic polymer without an effective positive charge; and b) recovering nucleic acid from the matrix.

Brief Summary Text (25):

The invention also includes an apparatus for isolating nucleic acid in a substantially purified form from multiple biological samples simultaneously, the apparatus comprising: plural housings for isolation of nucleic acid from plural biological samples, wherein each housing comprises an inlet and an outlet and defines a flowpath for flow of a biological sample therethrough, the flowpath comprising a matrix comprising a solid hydrophilic organic polymer without a net positive charge, and support means for holding plural housings in place such that nucleic acid in plural

biological samples may be handled and isolated simultaneously.

Brief Summary Text (28):

The nucleic acid isolated as described herein may be of any molecular weight and in single-stranded or double-stranded form; i.e., small oligonucleotides such as 10-50 bases in length, small nucleic acid fragments of, for example, 100 bases-500 bases in length, or relatively longer fragments of 1000 bases-10,000 bases in length. Alternatively, high molecular weight nucleic acid, e.g., 50 kb-500 kb may be isolated as described herein. Preferably, a nucleic acid isolated according to the invention will be in the range of 100 bases to 100 kilobases.

Detailed Description Text (2):

The invention encompasses a method and apparatus for nucleic acid isolation and concentration, and takes advantage of the discovery that aggregated nucleic acid binds reversibly to a solid, hydrophilic organic matrix without an effective positive charge.

Detailed Description Text (4):

According to the invention, a nucleic acid aggregate is contacted with a solid, hydrophilic organic matrix without an effective positive charge under conditions and for a time sufficient to allow it to bind reversibly to the matrix. If desired, the matrix-nucleic acid complex may be washed to remove contaminants, then dissociated and/or solubilized, and nucleic acid recovered in water or low salt buffer. The method of the present invention permits the investigator or technician to isolate nucleic acid of essentially any molecular weight in a rapid, high-yield manner.

Detailed Description Text (6):

Solid hydrophilic organic polymers that constitute a matrix useful according to the invention fall within the definition provided hereinabove. A matrix according to the invention will include any solid, hydrophilic organic matrix without an effective positive charge that reversibly binds nucleic acid through Van der Waals interactions and not by electrostatic interactions, affinity binding, or physical trapping. Preferably, the matrix is essentially neutral, i.e., without any positive or negative charge.

Detailed Description Text (7):

The term "solid matrix", as used herein, encompasses a polymer that is substantially insoluble in water and alcohol at less than about 50 degrees centigrade. Preferably, a solid matrix is in particulate form, with the particles being in the micro-meter range (preferably, 5-500 .mu.meters) or the milli-meter range (preferably, 0.1-10 mmeters); or is in fibrous form with the fibers being micro-meter in diameter and of any desired length.

Detailed Description Text (18):

Tissues or cells that contain nucleic acids are suspended in an extraction solution that contains a buffer system, a detergent, and a chelating agent. The buffer system can be any buffer, e.g., TrisHCl, sufficient to maintain pH values from approximately 5.0 to approximately 10. The detergent can be ionic or nonionic detergent, such as sodium dodecyl sulfate (SDS) or octylglucoside, at a concentration sufficient to lyse cells and denature proteins. A chelating agent, such as EDTA, captures free divalent ions (Mg.sup.2+) so that nucleic acids are more soluble and protected from degradation by DNA-degrading enzymes that require Mg.sup.2+.

Detailed Description Text (20):

Nucleic acid aggregates are next formed by precipitating the nucleic acid. Structurally, nucleic acid possesses a phosphodiester backbone that is negatively charged around neutral pH. Nucleic acid becomes insoluble (i.e., precipitated) in the presence of salts and agents that can reorganize its aqueous environment. Acetone, alcohols such as ethanol and isopropanol, and soluble organic polymers, such as polyethylene glycol (PEG) are examples of useful precipitants. Co-precipitants, such as glycogen, also may be used to facilitate the precipitation of nucleic acid present in only small quantities; for example, in the form of heteroaggregates. The presence of a co-precipitant is not required according to the invention, but serves to increase the efficiency of aggregate formation. In the presence of moderate concentrations of monovalent salt, the charges in nucleic acids are neutralized so that nucleic acid

aggregates are formed and stabilized by Van der Waals forces. Divalent salts such as magnesium chloride or calcium chloride can also be used to precipitate nucleic acids.

Detailed Description Text (52):

Solutions and reagents are the same as those used in Example 1 unless otherwise specified. In addition, 1 M MgCl₂ and chelating resin are used. Column matrix and columns are prepared as in Example 1 unless otherwise specified.

Detailed Description Text (53):

4 full circles (15 mm diameter) of dried blood spots are removed from S&S 903 filter papers. Two of them are immersed in 5 ml of extraction buffer (Sample 1) and the other two are placed in 5 ml of the same buffer plus 0.1 g chelating resin (Sample 2). The samples are incubated at 56.degree. C. for about 2 hours to digest proteins by proteinase K.

Detailed Description Text (67):

The same amount of nucleic acid (mainly DNA) is recovered by the column method as by the control method, based on gel electrophoresis analysis. Samples treated with chelating resins contained DNAs of relatively large molecular weights.

Detailed Description Text (82):

To purify single stranded plasmid DNA, the following steps are performed:

Detailed Description Text (90):

The results demonstrate that similar amounts of double-stranded plasmid DNAs are isolated with cellulose matrix as with a silica matrix. Single-stranded plasmid DNAs are also isolated, although the amount of DNA isolated by the cellulose matrix method is slightly less than that isolated after phenol extraction and ethanol precipitation. DNAs isolated by the cellulose matrix method are sequenced as efficiently as DNAs isolated by phenol extraction and ethanol precipitation method. Plasmid isolation by this method of the invention eliminates the use of chaotropic agents and minimizes the use of high speed centrifugation.